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THE ETIOLOGY OF PYEMIC ARTHRITIS IN FOALS *

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This disease is common to calves and foals, occurring generally when the animal is but a few days old, altho typical cases may occur in foals at the age of several months. The symptoms consist of rapidly developing articular inflammation causing impaired locomotion, fever, omphalophlebitis and often pulmonary and intestinal disturbances. Suppuration of infected joints with internal abscesses is common.

Lack of definite knowledge in regard to the etiology is apparent when one studies the literature. The earliest description of the disease was by Bernard of Boulogne in 1828. The symptoms are accurately recorded and it is interesting that he believed the disease originated in the mares and was transmitted through the milk. A few years later, Prichard, an English writer, after describing the disease, states the cause to be due to an alteration in the milk, the result of working the mare and allowing the colt to suckle between periods. Other early writers considered the disease as tuberculous or as rheumatism. To-day most of those who have studied the disease believe that it results from umbilical infection at the time of birth or subsequently. This view has much evidence in its support, but altho it has been held for years and appropriate precautions taken to prevent infection through this channel, the disease is still extremely prevalent. Cases also develop in utero or apparently a few hours after birth. At present there is no unity of opinion as to the causative agent.

PERSONAL OBSERVATIONS

During the last two years many bacteriological examinations have been made of material from cases of this disease forwarded to this laboratory. In most cases practitioners would send only pus formed in the joints during the latter period of the disease and in almost all such cases the cultures showed mixed infection. That the organism to be discussed was not recovered earlier is, I think, accounted for in this

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way. On several occasions, however, I have obtained fluid from infected joints during the early days of the infection, and in this fluid the organism to be described was obtained in pure culture.

Case 1.—Colt foaled in open; disease never observed on the premises before altho three cases occurred this season; navel not ligatured at time of birth. When 8 days old developed typical case of "joint ill," the left hock being chiefly involved. Ten days after first symptoms the hock was aspirated and cultures made of the synovia; at the same time blood cultures were made from the jugular on glucose liver broth.

On the blood agar inoculated with the synovia a pure culture of a gram-negative bacillus was obtained; eight colonies developed, all of the same type. Direct smear showed a few leukocytes but no bacteria. This, however, is not strange as the inflammation is chiefly periarticular.

In eighteen hours large quantities of gas formed in the glucose broth culture from the blood. Smears showed numerous gram-negative bacilli. On plating out, a pure culture was obtained of the same organism as that from the synovia. This animal died about four weeks later and at that time I isolated streptococci as well as gram-negative bacilli, the streptococci predominating.

Case 2.—Colt, foaled in the open; navel ligatured by veterinarian and treated antiseptically. Twelve days after birth typical symptoms of joint ill were observed. Ten days later the affected hock was aspirated and cultures made from synovial fluid, blood cultures being made at the same time. From the joint on blood agar a pure culture of a gram-negative bacillus was obtained; again the number of colonies were few, about twelve, all of them having the same appearance. From the blood pure culture of an organism identical with the one obtained from the synovia was isolated.

Case 3.—Colt born in open, navel not ligatured, no evidence of umbilical infection. Ten days after birth joint ill developed, both hocks being involved, giving pure cultures of a gram-negative bacillus. This colt made a slow recovery.

Case 4.—In this case no history was given save that the sample of synovia has been aspired two hours after death. Numerous short gram-negative bacilli with many short-chain streptococci were present in the smears. From blood agar plates both organisms were isolated.

Case 5.—No history, fluid aspirated from joint after death. The smears gave gram-positive cocci and gram-negative bacilli. Culture on agar gave a growth of the staphylococcus aureus, streptococci and colonies of a gram-negative bacillus.

Case 6.—No history; blood culture sent with statement that it was from a case of septic arthritis. Blood agar plates gave pure growth of a gram-negative bacillus.

In two other typical cases, pure cultures of streptococcus were obtained from the pyemic joints. In one case this organism was obtained from the blood also.

Pus from about fifteen discharging joints and navels has been examined and all showed mixed infection with streptococci, staphylococcus aureus and colon bacilli, streptococci generally predominating.

MORPHOLOGICAL AND CULTURAL CHARACTERISTICS OF THE BACILLUS

It is a very short bacillus with rounded ends frequently resembling a coccus, from .2 to .5 micron in width, and .3 to 1 micron in length. In old cultures much longer organisms are often observed; frequently seen in pairs, but never in chains. It is gram-negative, stains well with all anilin dyes, and very motile, especially in young broth cultures.

Agar Slants.—On agar + 6 to + 1.0 to phenolphthalein the growth, especially when blood has been streaked over the surface, is characteristic within twenty-four hours. The growth is dull and if it has spread to water of condensation, this is covered with a grayish pellicle; at the base of the slant numerous wrinkles develop especially in the median line. The growth is not slimy but more membranous, being removed from the slant at times with difficulty.

TABLE 1

THE RESULTS OF TESTS OF CARBOHYDRATE FERMENTATION

Strain	Glucose	Lactose	Mannite	Dulcite	Raffinose	Saccharose	Milk
Blood, Case 1	A+ G+	A— G—	A+ G+	A+ G	A— G—	A— G—	A— G—
Joint, Case 1	A+ G+	One bubble on 2d day	A+ G+	A+ G	A— G—	A— G—	A— G—
Joint, Case 1 at death	A+ G+	A— G—	A+ G+	A+ G	A— G—	A— G—	A— G—
Joint, Case 2	A+ G+	One bubble on 2d day	A+ G+	A+ G	A— G—	A— G—	A— G—
Blood, Case 2	A+ G+	A— G—	A+ G+	A+ G	A— G—	A— G—	A— G—
Joint, Case 3	A+ G+	A— G—	A+ G+	A+ G	A— G—	A— G—	A— G—
Joint, Case 4	A+ G+	A— G—	A+ G+	A+ G	A— G—	A— G—	A— G—
B. coli communis "Dunham"	A+ G+	A+ G+	A+ G+	A+ G	A+ G+	A+ G+	A+ G+

A+ = acid produced. A— = no acid produced. G+ = gas produced. G— = no gas produced.

Agar Streak.—This is also characteristic after a few days' growth. Where the growth is most luxuriant at the bottom of the slant there is a tendency to spread out in concentric rings through which radiate numerous wrinkles.

Agar Plates.—At first the colonies are transparent and delicate, but in twenty-four hours a peculiar concentric ring formation occurs.

Gelatin Stab.—No liquefaction.

Milk.—Changed in reaction after five days from plus 1.4 to phenolphthalein to plus 0.9; alkaline to litmus.

Broth.—For first twenty-four hours the growth is very turbid; later some strains produce a pellicle while others do not. In both cases a slightly viscid sediment is produced.

Peptone Solution.—No indol.

Nitrate Broth.—Not reduced.

Carbohydrate Fermentation.—The media were prepared according to the standard methods of the American Public Health Association. The broth was incubated for four days. The results are given in Table 1.

From these characteristics it is seen that the organism belongs to the colon-typhoid group and is closely related to *B. paratyphosus beta.* To further ascertain this relationship, complement fixation tests were made, using antigens prepared from two strains of the paratyphoid bacillus B., the organism under discussion and colon bacilli. The antigens were made as described on page 5 and their strengths equalized as nearly as possible. Fixation of complement was never obtained with these antigens, altho controls both positive and negative were quite satisfactory. Altho shown by these tests not to be a true paratyphoid bacillus B., the organism can, I think, be considered as of a closely related species.

AGGLUTINATION

A potent agglutinative serum was obtained by injecting a rabbit intravenously on three successive days with 50 million dead organisms (Strain 4). On the sixth day after the last injection the rabbit was bled, serum separated and inactivated at 56 C. for thirty minutes. It now caused agglutination of Strain 4 in a dilution of 1 to 3,000. Table 2 shows the results of these tests. The different strains were grown for twenty-four hours at 37 C. and broth + 10 to phenolphthalein diluted to a uniform turbidity was used as in the typhoid agglutination test.

TABLE 2
AGGLUTINATION

Strain	Dilution of Immune Serum			
	$\frac{1}{1500}$	$\frac{1}{2000}$	$\frac{1}{3000}$	$\frac{1}{4000}$
1	++	++	±	—
2	++	++	±	—
3	++	++	++	±
4	++	++	++	—
5	++	±	±	—
6	++	++	±	—
7	++	++	±	—

++ = strong agglutination. ± = doubtful agglutination. — = no agglutination.
Normal rabbit serum in no case agglutinated in dilutions above 1 to 10.

The tests, if not demonstrating the absolute similarity of these organisms, indicate their close relationship, the results being far more pronounced than those in group reactions.

COMPLEMENT FIXATION

Experiments were made to establish if possible relationship between the organism and the disease. The time of the year (November) was unfortunate, as no acute cases could be obtained and only few chronic ones. There were, however, some cured cases and the blood was obtained from them, also from their dams and from mares that had given birth to foals which developed the disease.

TABLE 3

TITRATION OF COMPLEMENT AND HEMOLYSIN

5 Percent Suspension of Sheep Cells	Hemolysin 1/1000	Complement 1/10	Normal Salt Solution	Hemolysis
.5 c.c.	.25	.25	1.5	Complete
.5 c.c.	.2	.25	1.55	Complete
.5 c.c.	.15	.25	1.6	Complete
.5 c.c.	.1	.25	1.65	Partial
.5 c.c.	.05	.25	1.7	None
.5 c.c.	.25	.2	1.55	Complete
.5 c.c.	.2	.2	1.6	Partial
.5 c.c.	.15	.2	1.65	Partial
.5 c.c.	.1	—.20	1.7	None
.5 c.c.	0	0	2	None
.5 c.c.	.5	0	2	None
.5 c.c.	0	.5	1.5	None

Hemolysin .15 = 1 unit of hemolysin with .25 complement; in test .3 hemolysin would be used with .5 complement. The quantities in Tables 3, 4 and 5 refer to c.c.

Agar slants were grown for twenty-four hours, the organisms washed off in normal salt solution, the suspension heated at 60 C. for fifteen minutes, carbolic acid added to make 0.5 percent strength. Antigen was tested for specificity by using normal and immune rabbit serum and normal horse-serum. Complement was obtained in the usual way from fresh guinea-pig blood, diluted to one-tenth, then titrated. The serum to be tested was removed from the clot and inactivated at 56 C. for one-half hour within forty-eight hours after clotting. The night before the test was made sheep blood-cells (2 drops solid cells) were added to each tube and kept in ice-chest. This has been found the simplest way to avoid inaccuracy due to presence of natural hemolysin. Tables 3, 4 and 5 illustrate the methods used.

In some cases fixation could be obtained when much greater dilutions of antigen and serum were used. It must, however, be borne in mind that the animals from which the blood was obtained were either cured cases or apparently normal animals. On this account the concentration of antigen and serum in the tests was increased, altho not sufficiently to make the test of doubtful value.

TABLE 4
TITRATION OF ANTIGEN

Antigen	Serum	Complement	Normal Salt Solution	Hemolytic Amboceptor	Sheep Cells 4 Percent Suspension	Hemolysis
1.0 c.c.	Normal .15 c.c.	.5	—	.5	.5	Complete
.8 c.c.	Normal .15 c.c.	.5	.05	.5	.5	Complete
.5 c.c.	Normal .15 c.c.	.5	.35	.5	.5	Complete
0	Normal .3 c.c.	.5	.7	.5	.5	Complete
1.0 c.c.	—	.25	.25	.5	.5	Complete
1.0 c.c.	—	.5	.5	.5	.5	None
.5 c.c.	Immune .1	.5	.4	.5	.5	None
.4 c.c.	Immune .1	.5	.5	.5	.5	None
.3 c.c.	Immune .1	.5	.6	.5	.5	None
.2 c.c.	Immune .1	.5	.7	.5	.5	Trace
.1 c.c.	Immune .1	.5	.8	.5	.5	Almost Complete
—	Immune .2	.5	.8	.5	.5	Complete

Antigen, serum, complement and salt solution at 37 C. (water bath) for 30 minutes before adding amboceptor and sheep corpuscles. 0.5 per cent. antigen was maximum quantity used; 2½ times that quantity which gave complete fixation with known positive serum. This quantity was not anticomplementary nor was it hemolytic.

TABLE 5
ILLUSTRATION OF COMPLEMENT FIXATION TEST

Antigen	Serum	Complement 2 Units	Salt Solution	Hemolysin 2 units	Cells	Hemolysis
.5	Known positive .15	.5	.35	.5	.5	None
.5	Known positive .1	.5	.4	.5	.5	None
.5	Known positive .05	.5	.45	.5	.5	None
.0	Known positive .3	.5	.7	.5	.5	Complete
.5	Known negative .15	.5	.35	.5	.5	Complete
.5	Known negative .10	.5	.4	.5	.5	Complete
.5	Known negative .05	.5	.45	.5	.5	Complete
.0	Known negative .3	.5	.7	.5	.5	Complete
.5	Serum to be tested .15	.5	.35	.5	.5	None
.5	Serum to be tested .10	.5	.4	.5	.5	None
.5	Serum to be tested .05	.5	.45	.5	.5	Partial
.0	Serum to be tested .3	.5	.7	.5	.5	Complete

The results obtained from a considerable number of tests (Table 6) indicate that a specific relationship exists between the organism isolated and used as antigen and the disease pyemic arthritis or joint ill as it occurs in this region. Certain cases (2, 3, 15, 16, 17, 18) are especially interesting, positive reactions being obtained from the blood of both dam and foal. Numbers 8, 9, 25 and 29 also are worthy of special notice, these mares having aborted during the year, and it will be noticed, gave a certain amount of fixation indicating a possible relationship between the organism isolated and that of contagious equine abortion.

TABLE 6

RESULTS OF TESTS FOR COMPLEMENT FIXATION

1.	Normal horse	No fixation
2.	Dam in Case 3	Complete fixation
3.	Foal in Case 3	Complete fixation
4.	Dam in Case 4	Complete fixation
5.	Gelding, 12 years, on infected farm	Complete fixation
6.	Mare, 5 years, had arthritis as foal	No fixation
7.	Dam of diseased foal	Partial fixation
8.	Mare, aborted in spring	Complete fixation
9.	Mare, aborted in spring	Complete fixation
10.	Colt recovered from joint ill	Complete fixation
11.	Mare, history unknown	No fixation
12-13.	Geldings, history unknown	No fixation
14.	Immunized rabbit	Complete fixation
15.	Mare, foal with joint ill in spring	Complete fixation
16.	Colt of Mare 15	Complete fixation
17.	Mare, foal joint ill in spring	Complete fixation
18.	Foal of Mare 17	Complete fixation
19.	Colt, 2 years, pyemic	Complete fixation
20-24.	Mares giving birth to foals with joint ill this spring	Complete fixation
25.	Mare, aborted this spring	Almost complete fixation
26.	Mare, never in foal, joint ill on farm	Almost complete fixation
27.	Mare, had foal with joint ill all this spring	Almost complete fixation
28.	Colt, joint ill one year ago	50 percent hemolysis
29.	Mare, aborted two months ago	50 percent hemolysis
30-46.	Mares giving birth to foals with joint ill this spring	No fixation
46-52.	Mares that recently aborted	No fixation
38-39.	Foals recovered from joint ill	No fixation
40-50.	Supposedly healthy mares	No fixation

Cases 20-50 occurred in a district where septic arthritis has been prevalent for years.

DISCUSSION

To what extent the organism described is responsible for septic arthritis cannot as yet be stated. Isolating the organism from blood and joint in pure culture from acute cases, altho suggestive, by no means demonstrates that it possesses pathogenic properties for the animal from which it was recovered. But the fact that positive fixation tests have been obtained frequently with blood of diseased foals and their dams and not from the healthy controls is strong evidence in favor of a definite relationship between the organism and certain cases of arthritis. Further, I think that these results strongly support the idea that the foal frequently is infected before birth. Intra-uterine infection alone can account for the cases that develop within a few hours after birth. I would not wish to belittle the present prophylactic methods, but rather supplement them, and thereby prevent infection from a hitherto neglected source. In many instances arthritis no doubt may arise from navel infection with the usual pus-forming bacteria subsequent to birth; but as before stated, such infection cannot account for all cases. The possibility of infection being carried from diseased to healthy mares by coitus must not be overlooked.

I would like to draw attention to the interesting fact that the organism isolated and described in this paper bears a very close relationship to one recently isolated by Good in contagious equine abortion. In cultures the only difference observed is that the bacillus of equine abortion causes fermentation in raffinose while my organism does not attack this carbohydrate. However, difference in strains would readily account for this. The most convincing evidence that these organisms are but strains of same species is that they cannot be differentiated by means of the complement fixation test. Serums giving complete fixation with antigen prepared from my organism also give fixation in corresponding dilutions with antigen prepared from the bacillus of equine abortion. With characteristics that correspond so closely, these organisms appear to be but strains of the same species and it is quite probable that the same organism may be responsible for both septic arthritis and abortion. These diseases commonly co-exist in the same locality; furthermore, it is of frequent occurrence for a mare to abort one season and during the next to deliver a colt that develops septic arthritis. The more one studies the clinical history of these diseases the more convincing becomes the probability of their common etiology in many instances.